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Systematics and phylogeography of the *Dysdera erythrina* species complex (Araneae, Dysderidae) in Sardinia

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Abstract

Sardinia is the second largest island in the Mediterranean and, together with Corsica and nearby mainland areas, one of the top biodiversity hotspots in the region. The origin of Sardinia goes back to the opening of the western Mediterranean in the late Oligocene. This geological event and the subsequent Messinian Salinity Crisis and Pleistocene glacial cycles have had a major impact on local biodiversity. The *Dysdera* woodlouse hunter spiders are one of the most diverse ground-dweller groups in the Mediterranean. Here we describe the first two species of this genus endemic to Sardinia: *Dysdera jana* **sp. n.** and *Dysdera shardana* **sp. n.** The two species show contrasting allopatric distribution: *D. jana* **sp. n.** is a narrow endemic while *D. shardana* **sp. n.** is distributed throughout most of the island. A multi-gene DNA sequence phylogenetic analysis based on mitochondrial and nuclear genes supports the close relationships of the new species to the type species of the genus *Dysdera erythrina*. Age estimates reject an Oligocene origin of the new *Dysdera* species and identify the Messinian Salinity Crises as the most plausible period for the split between Sardinian endemics and their closest relatives. Phylogeographic analysis reveals deep genetic divergences and population structure in *Dysdera shardana* **sp. n.**, suggesting that restriction to gene flow, probably due to environmental factors, could explain local speciation events.

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Keywords
Taxonomy, phylogeny, DNA sequencing, Mediterranean biogeography, phylogeography

Introduction

The Mediterranean basin is one of the Earth's biodiversity hotspots for conservation priority (Myers et al. 2000). A dynamic and tumultuous geological history ranks high among the main factors responsible for the outstanding diversity of the region. The island of Sardinia constitutes a good example of this ever-changing geological landscape. At the beginning of the Oligocene (~30 million years ago, m.a.), several continental microplates, including present-day Sardinia, Corsica, the Balearic Islands, Calabria, the Kabylies and the Betic-Rift Cordillera, broke off from the north-eastern Iberian Peninsula as a result of geological back-arc extension processes, and started drifting to their present-day location. During the first stage of the back-arc extension, the northern microplate assemblage (i.e., Sardinia, Corsica and Calabria) started drifting counter-clockwise with respect to the Eurasian plate and finally collided with the Apulian plate about 20-18 m.a. (Rosenbaum et al. 2002). There is no general consensus on the time of separation of Corsica and Sardinia. Some authors suggest that Sardinia broke off from Corsica 20 to 16 m.a. (Rosenbaum et al. 2002); while others consider that the process would not have been fully completed until about 9 m.a. (Alvarez 1972, 1974). The closing of the Miocene marine gateways between the Mediterranean Sea and the Atlantic Ocean, 5.96 m.a., caused a large drop in the Mediterranean water level (Blondel and Aronson 1999; Krijgsman et al. 1999) and the emergence of land connections between Sardinia and northern Italy and southern France. The opening of the Strait of Gibraltar about 5.3 m.a. restored the water exchange between the Atlantic and Mediterranean basins, re-establishing effective isolation of the island ecosystems (Loget and Van Den Driessche 2006; Loget et al. 2005). Eustatic sea level changes as a result of Pleistocene glacial cycles could have connected Sardinia with Corsica, and with the mainland, via Elba. Sea level oscillations creating land bridges between Sardinia and Corsica from the Miocene until well into the Pleistocene (5.7 – 0.23 m.a.) have been additionally suggested (Arias et al. 1980).

Sardinia is the second largest island in the Mediterranean (Fig. 1), generally mountainous with a few coastal plains. The island's mountains are divided into three ranges that largely coincide with areas of high endemism, namely Mount Limbara on the north, the Gennargentu-Supramonte Massif on the central-eastern side, and the Sette Fratelli on the south-east (Grill et al. 2007). Additionally, a separate biogeographic region has been proposed for the Sulcis-Iglesiente territory in the south-western part of Sardinia, based on exclusive plant endemism and a unique blend of geolithologic, geomorphologic, paleogeographic, bioclimatic and vegetation features (Bacchetta et al. 2007). It is worth noting that the Tyrrhenian islands, Sardinia and Corsica, and the Maritime-Ligurian Alps, are among the regions with the highest level of narrow endemism in the Mediterranean basin (Medail and Quezel 1999).

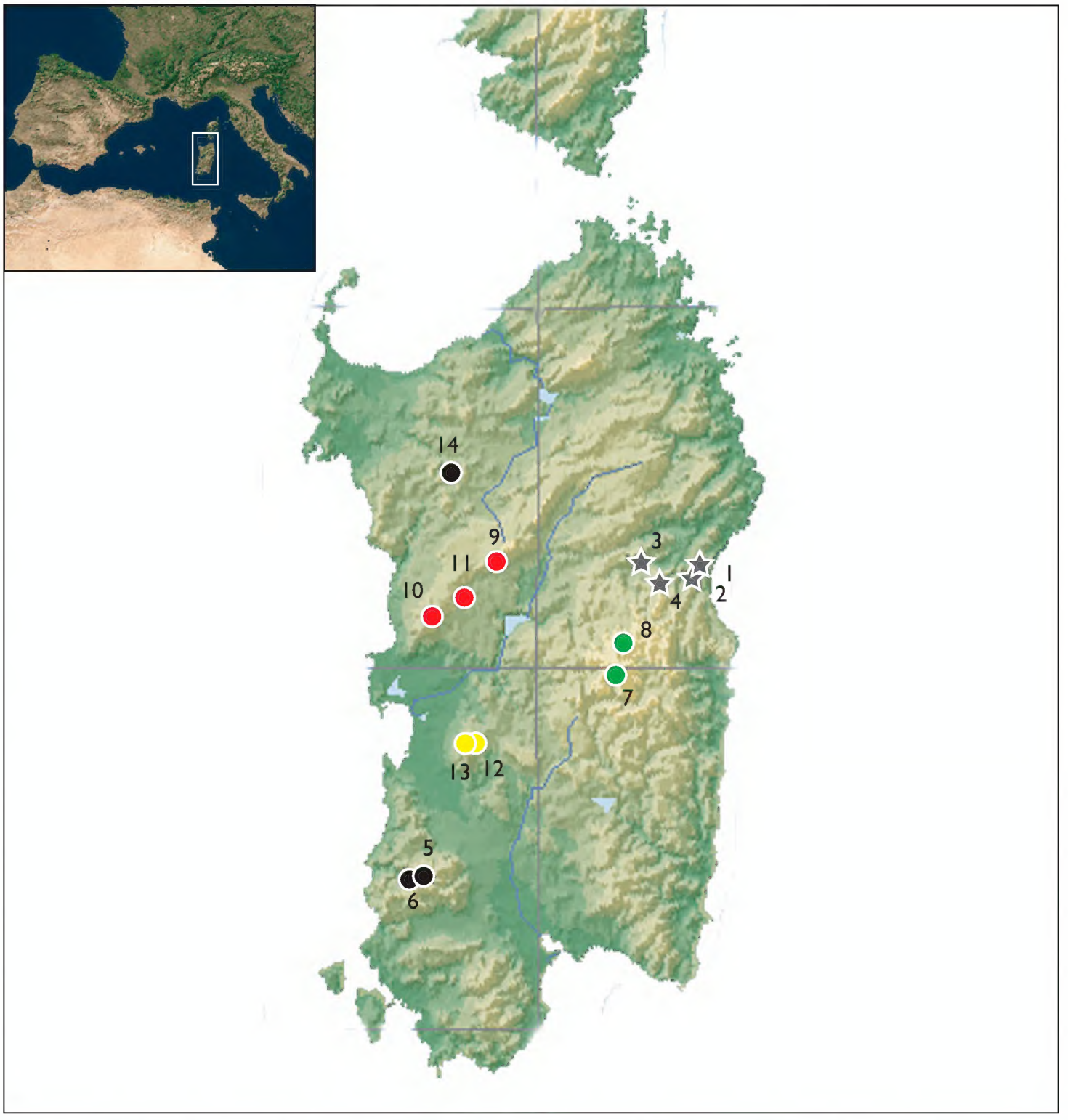


Figure 1. Maps showing the location of Sardinia and known localities of the new species. *Dysdera jana* sp. n.: Nuoro: **1** Dorgali, 450 m, **2** 2 km S of Dorgali, 500 m, **3** Supramonte di Oliena, 900 m, **4** Sa Oche, 7 km ESE of Oliena, 150 m. *D. shardana* sp. n.: Cagliari: **5** Fluminimaggiore, Is Arenas, **6** Fluminimaggiore, Tempio di Antas, Nuoro: **7** Aritzo, Castagneto Geratzia, 867 m, **8** Desulo, rd. to Fonni, 1146 m, **9** Macomer, Mt. S. Antonio. Oristano: **10** Cuglieri, rd. to San Leonardo 7 Fuentes, 587 m, **11** S. Leonardo 7 Fuentes, 699 m, **12** Morgongiori, Is Benas recreation area, 566 m, **13** Morgongiori, Bruncu Mutzuis, 761 m, **14** Sassari: NW Torralba.

The woodlouse-hunter spiders of the genus *Dysdera* Latreille, 1804 include about 250 described species (Platnick 2009) of mostly nocturnal, wandering, ground-dwelling spiders. Although the geographic distribution of the genus includes most of the Western Palaearctic, it is especially diverse in the Mediterranean region, where it constitutes one of the most conspicuous components of the local ground arthropod fauna. The *erythrina* group was originally proposed by Deeleman-Reinhold and Deeleman

(1988) to include the type species of the genus, *Dysdera erythrina* (Walckenaer, 1802), as well as some other species described from the Iberian peninsula, which included *D. fuscipes* Simon, 1882, *D. anonyma* Ferrández, 1984 and *D. veigai* Ferrández, 1984. This group was proposed to be related to the *crocata* group, from which it would differ by the shape and size of the carapace and the chelicera, the spination pattern and the shape of the male bulb (male secondary copulatory structure, located on palpal tarsi). The great French arachnologist E. Simon described three subspecies of *D. erythrina*, namely *D. erythrina lantosquensis* Simon, 1882 from France, *D. erythrina fervida* Simon, 1882 from Corsica and the Balearic Islands and *D. erythrina provincialis* Simon, 1882 from France (Platnick 2009). Subsequently, Řezáč and collaborators (2007) have granted species status to *D. lantosquensis*, and suggested that the rest of the subspecies should also be considered different species.

Sardinia harbours a rich endemic Dysderidae fauna, which includes four *Harpactea* Bristowe, 1939 species, one *Parachtes* Alicata, 1964 and the monotypic cave-dwelling endemic genus *Sardostalita* Gasparo, 1999 (Platnick 2009; Stoch 2003). Endemic *Dysdera* species have not been reported from Sardinia so far. The *crocata* group is represented by the synanthropic *D. crocata* C.L. Koch, 1839, *D. ancora* Grasshoff, 1959 and *D. ventricosa* Grasshoff, 1959, the latter two species also present in Sicily (Stoch 2003). Grasshoff (1959) reported the presence in Sardinia of *D. kollari* Doblika, 1853, a species previously reported from the eastern Adriatic coast (Chyzer and Kulczyński 1897) and northern Greece, and described the formerly unknown male (Grasshoff 1959) (it turned out to be a misidentification, see below; a drawing of the actual *D. kollari* male palp can be found in Gasparo 2004). However, the former citation has been suggested to refer to another species (Deeleman-Reinhold and Deeleman 1988). Finally, there is an early record of the presence of *D. erythrina* from Sardina (Costa 1885), which has not been subsequently confirmed to date.

In the present paper we confirm the presence of the *erythrina* group in Sardinia based on museum collections and newly collected material, and describe two new species, one of them including specimens previously misidentified as *D. kollari*. Phylogenetic position, time of origin and phylogeographic patterns of the new species are further investigated by means of molecular evidence.

Methods

Taxonomy

Morphological methods are described in detail in Arnedo and Ribera (1999) and Arnedo et al. (2000). Taxonomic descriptions follow the format of Arnedo and Ribera (1999). Specimens were examined using a Leica MZ16A stereoscopic microscope equipped with a Nikon DXM1200 digital camera and an ocular measuring graticule. Digital microscope images were edited using the Auto-Montage software package v.4 (Syncroscopy, Frederick, MD, USA)]. Male right palps were removed, cleaned by means of ultrasound

and examined using either a HITACHI S-2300 Scanning Electron Microscope (SEM) (SCT, Universitat de Barcelona, Barcelona, Spain) operated at 15 kV. Left male palps were used for optic images and right palps were used for SEM images. All morphological measurements are given in millimetres. Eye diameters were taken from the spans of the lens. Carapace and abdomen measurements were taken in dorsal view, abdomen hairs were measured in lateral view, and cheliceral basal segment length was measured in lateral view. The prolateral groove of the chelicera was measured in dorsal view, by positioning the chelicera parallel to the background and measuring from the distal end of the margin to the beginning of the cheliceral lamina. The fang was measured in ventral view, from the basal segment condyl to the fang distal tip. The largest leg article lengths were measured in lateral view without detaching the legs from the specimen, by placing the article being measured in a perpendicular position. The female vulva was removed and muscle tissues were digested using a KOH (35%) solution before observation. Leg spination was recorded using the codification method fully described in Arnedo and Ribera (1997).

Abbreviations used in the text and figures are as follows:

Eyes

AME anterior medial eyes;
PME posterior medial eyes
PLE posterior lateral eyes

Male copulatory bulb

T tegulum
DD distal division
IS internal sclerite
ES external sclerite
C crest
AC additional crest
AR arch-like ridge
LF lateral fold over lateral sheet between internal and external sclerites
L lateral sheet
LA lateral sheet anterior apophysis
AL additional lateral sheet at the internal border
P posterior apophysis

Female genitalia

DA dorsal arch
DF dorsal arch fold
S spermatheca
TB transversal bar

Repositories

CRBA Centre de Recursos de Biologia Animal, University of Barcelona
CG F. Gasparo personal collection.
MSNVR Museo Civico di Storia Naturale di Verona (P. Brignoli's collection)

Molecular phylogeny

Taxa analysed in the present study, along with locality information and the GenBank accession numbers of the sequenced genes are listed in Table 1. Fresh specimens of *D. jana* sp. n. were not available for DNA analysis. Phylogenetic relationships of *D. shardana* sp. n. were inferred from a taxonomic sample that included all subspecies of *Dysdera erythrina*, representatives of the *erythrina* group (*D.* cf. *inermis*), putative related species based on genitalia characteristics (*D. lusitanica* Kulczyński, 1915, *D. valentina* Ribera, 2004), as well as two members of the *crocata* group. *D. adriatica* Kulczyński, in Chyzer and Kulczyński, 1897 was used as an outgroup based on the results of Macías-Hernández et al. (2008). The inclusion of *D.* cf. *inermis* specimens from both sides of the Strait of Gibraltar provided a vicariant biogeographic point (opening of the strait 5.3 million years ago) to estimate lineage age, by assuming that the separation of Iberian and Moroccan populations of *D.* cf. *inermis* was the result of this geological event. The same assumption has been used to calibrate molecular phylogenies in Canarian *Dysdera* (Macías-Hernández et al. 2008), scorpions (Gantenbein 2004), beetles (Gómez-Zurita 2004) and newts (Carranza and Arnold 2003). A second data matrix was built to investigate phylogeographic patterns in *D. shardana* sp. n. by sampling all freshly collected specimens amenable to DNA analyses, namely 14 specimens from localities in central Sardinia (Mt. Ferru, Mt. Arci, and Gennargentu).

Protocols for specimen handling, DNA extraction, amplification, and sequencing of the gene fragments followed those of Macías-Hernández et al. (2008). The following gene fragments were analyzed: mitochondrial genes cytocrome *c* oxidase subunit I (*cox1*, 676 bp), 16S rRNA (16S), the complete tRNA leu UAG (L1) (16S+L1, 387-546 bp), NADH dehydrogenase subunit I (*nad1*, 362 bp), and the nuclear genes 28S rRNA (28S, 620-768 bp) and histone H3 (H3, 328 bp). Sequences were edited and handled using the computer program GENEIOUS v. 4.5 (Drummond et al. 2009). Alignment of the protein coding genes was trivial, since no length differences were observed and no indel events needed to be postulated. Sequences of 16S-L1 and 28S were automatically aligned using the online version of the program MAFFT v. 5.8 (Katoh et al. 2005; Katoh et al. 2002) (manual strategy set to G-INS-i). Gaps were subsequently recoded as separate presence/absence characters with the aid of the program GapCoder (Young and Healy 2003) following Simmons and Ochoterena (2000), to minimise the effect of increasing the weight of overlapping multiple non-homologous gaps (Pons and Vogler 2006). Gene partitions were combined in a single data matrix using WINCLADA v.1.00.08 (Nixon 2002), with non-sequenced fragments scored as missing data.

Parsimony analyses of the data matrices were conducted with the program TNT v. 1.1 (Goloboff et al. 2003), using the following search strategy: 1,000 iterations of Wagner trees constructed with random addition of taxa and subsequent TBR branch swapping, holding five trees per iteration up to a total maximum of 10,000. Clade support was assessed via jackknife resampling using 1,000 replicates with individual heuristic searches consisting of 15 iterations of Wagner tree construction using random

Table 1. Species included in the phylogenetic analysis and GenBank accession numbers for the gene sequences.

Species	Vouchers (DNA code)	Locality	*cox1*	16S-L1-*nad1*	28SrRNA	H3
Dysdera adriatica	CRBA1167	Slovenia, Kozina, Materija	EU068026	EU068064	GQ285610	GQ285620
Dysdera cf. *inermis*	NHM75	Spain, Tarifa, Mirador del Estrecho	EU068039	EU068073	EU139794	EU139725
Dysdera cf. *inermis*	NHM255	Morocco, Tanger	EF458142	EF458092	EU139795	EU139726
Dysdera crocata	CRBA851	Spain, Guadalajara, Hoz de Peregrina	EF458137	EF458095	GQ285611	GQ285621
Dysdera erythrina	CRBAVO70	Spain, Barcelona, Cardedeu,	GQ285643	GQ285603	GQ285619	-
Dysdera erythrina fervida	CRBA677	France, Iles d'Hyères	GQ285630	GQ285606/-	GQ285615	GQ285625
Dysdera erythrina provicialis	CRBAVO101	Spain, Barcelona, Montseny, Les Illes	GQ285642	GQ285602	GQ285618	-
Dysdera erythrina provicialis	K105	Spain, Barcelona, Sant Llorenç	AF244252	AF244162 /-	EU139790	EU139720
Dysdera lancerotensis	NMH441	Canary Is, Alegranza, la Caldera	EF458127	EF458080	EU139757	EU139686
Dysdera lantosquensis	CRBA653	Slovakia, Hrusov	GQ285628	GQ285604/-	GQ285612	GQ285622
Dysdera lantosquensis	CRBA678	Italia, Monte Argentario	GQ285631	GQ285607/-	GQ285616	GQ285626
Dysdera lusitanica	CRBA1002	Spain, Burgos, Puentedey	GQ285629	GQ285609	GQ285614	GQ285624
Dysdera shardana sp. n.	CRBA1238 (LB295)	Italy, Sardinia, Oristano, Cuglieri	GQ285635			
Dysdera shardana sp. n.	CRBA1240 (LB296), CRBA1708 (LB300)	Italy, Sardinia, Oristano, S. Leonardo 7 Fuentes	GQ285636			
Dysdera shardana sp. n.	CRBA1707 (LB299)	Italy, Sardinia, Oristano, S. Leonardo 7 Fuentes	GQ285637			
Dysdera shardana sp. n.	CRBA1247 (K477), CRBA1714 (LB297)	Italy, Sardinia, Orsitano, Morgongiori, Is Benas	GQ285633	GQ285605	GQ285613	GQ285623

Species	Vouchers (DNA code)	Locality	*cox1*	16S-L1-*nad1*	28SrRNA	H3
Dysdera shardana sp. n.	CRBA1256 (LB301)	Italy, Sardinia, Oristano, Morgongiori, Bruncu Mutzuis	GQ285638			
Dysdera shardana sp. n.	CRBA1262 (LB303), CRBA1711 (LB306)	Italy, Sardinia, Nuoro, Aritzo, Castagneto Geratzia	GQ285639			
Dysdera shardana sp. n.	CRBA1710 (LB304)	Italy, Sardinia, Nuoro, Aritzo, Castagneto Geratzia	GQ285640			
Dysdera shardana sp. n.	CRBA1263 (LB305), CRBA1712* (LB308)	Italy, Sardinia, Nuoro, Aritzo, Castagneto Geratzia	GQ285641			
Dysdera shardana sp. n.	CRBA1268 (LB294), CRBA1270 (LB307)	Italy, Sardinia, *Nuoro, Desulo, rd. to Fonni	GQ285634			
Dysdera valentina	CRBA777	Spain, Alacant, Cocentaina	GQ285632	GQ285608	GQ285617	GQ285627

addition of taxa, holding five trees per iteration and an overall maximum of 10,000. The program jMODELTEST v. 0.1.1 (Posada 2008) was used to select the substitution model that best fits the data with the fewest parameters (including branch lengths as parameters), as indicated by the Akaike information criterion (AIC) (Akaike 1973).

Bayesian inference analyses were conducted with MRBAYES v.3.1.2 (Ronquist and Huelsenbeck 2003) and run remotely at the University of Oslo's Bioportal computer resources (http://www.bioportal.uio.no). The substitution models suggested by jMODELTEST were specified for each gene fragment and a standard discrete model was implemented for the gaps scored as absence/presence data (Nylander et al. 2004). The substitution estimates were allowed to vary independently between each partition. Two independent runs with four simultaneous MCMC (Markov Chain Monte Carlo) chains (one cold and three heated), each starting with random starting trees, were carried out simultaneously, sampling 1,000 generations until the standard deviation of the split frequencies of these two runs dropped below 0.01 (10 million generations). The program TRACER v. 1.4 (Rambaut and Drummond 2003) was used to ensure that the Markov chains had reached stationary by examining the effective sample size (ESS) values and also to determine the correct number of generations to discard as a *burn-in* for the analysis.

Maximum likelihood searches were conducted with the stand-alone version of the computer program RAxML v. 7.0.3 (Stamatakis 2006). Individual models of nucleotide substitution (GTR+Γ+I, with default number of Γ-categories) were specified for each gene partition. The best trees were selected from 100 multiple inferences, and clade support was assessed by means of 100 non-parametric bootstrap resampled replicates of the original matrix.

Uncorrected genetic distances were estimated with MEGA *v.*4.0 (Tamura et al. 2007). A haplotype network was estimated from the *cox1* sequences using statistical parsimony, with probability connection limit set to 95%, as implemented in the program TCS v. 1.21 (Clement et al. 2000).

Lineage age estimates were obtained with the aid of the computer program R8S v. 1.71 (Sanderson 2003). Branch lengths were re-estimated with the computer program RAxML forcing the topology obtained in the Bayesian analysis and specifying independent GTR+I+G substitution models for each gene fragment. The same program was used to generate 100 trees with the same topology by bootstrapped branch lengths, to generate confidence intervals of the times of divergence. Trees were rooted and very short or zero branch length branches were pruned with the help of the software TreeEdit v. 1.0 (Rambaut and Charleston 2001). A preliminary cross-validation analysis (Sanderson 2002) conducted with r8s selected the penalized likelihood method with a smoothing parameter value of 3200 and additive penalty function as the best options for reconstructing divergence times in our data set. Subsequent analyses with the preferred method were conducted to obtain optimal ages and corresponding confidence intervals. The resulting chronograms were visualised and manipulated with the assistance of the computer program FigTree v. 1.1.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Results of the phylogenetic analyses conducted on all gene fragments concatenated in a single matrix are summarised in Fig. 2. Heuristic searches in TNT under equal weights yielded 3 trees of 2,015 steps. The best fit-models selected by the AIC for each data set were: TrN+I+G, TIM2+G, TIM2+G, GTR+G and TrNef+G for the *cox1*, 16S-L1, *nad1*, 28S and H3, respectively. All analyses support the sister group relationship of *D. shardana sp. n.* with the present and former subspecies of *D. erythrina*. All analyses support the hypothesis that *D. erythrina* sensu stricto and *D. erythrina provincialis* are sister species, while the sister-species relationship of *D. erythrina fervida* and *D. lantosquensis* is only contradicted by the maximum likelihood analysis, although with low support. *D. valentina* is resolved as the sister group to the clade formed by *D. shardana* sp. n. and *D. erythrina* sensu lato, although only the Bayesian analysis provides high support for this relationship. The phylogenetic analyses do not support a close relationship between either *D.* cf. *inermis* or *D. lusitanica* with the *erythrina* group. Parsimony partial analyses were run to assess the contribution of each data partition. The levels of support across the partial analyses were higher in the mitochondrial partition, followed by the 28S gene fragment, and the histone 3 was the less resolved. Instances of incongruence among the partial analyses were, in general, poorly supported. The mitochondrial genes

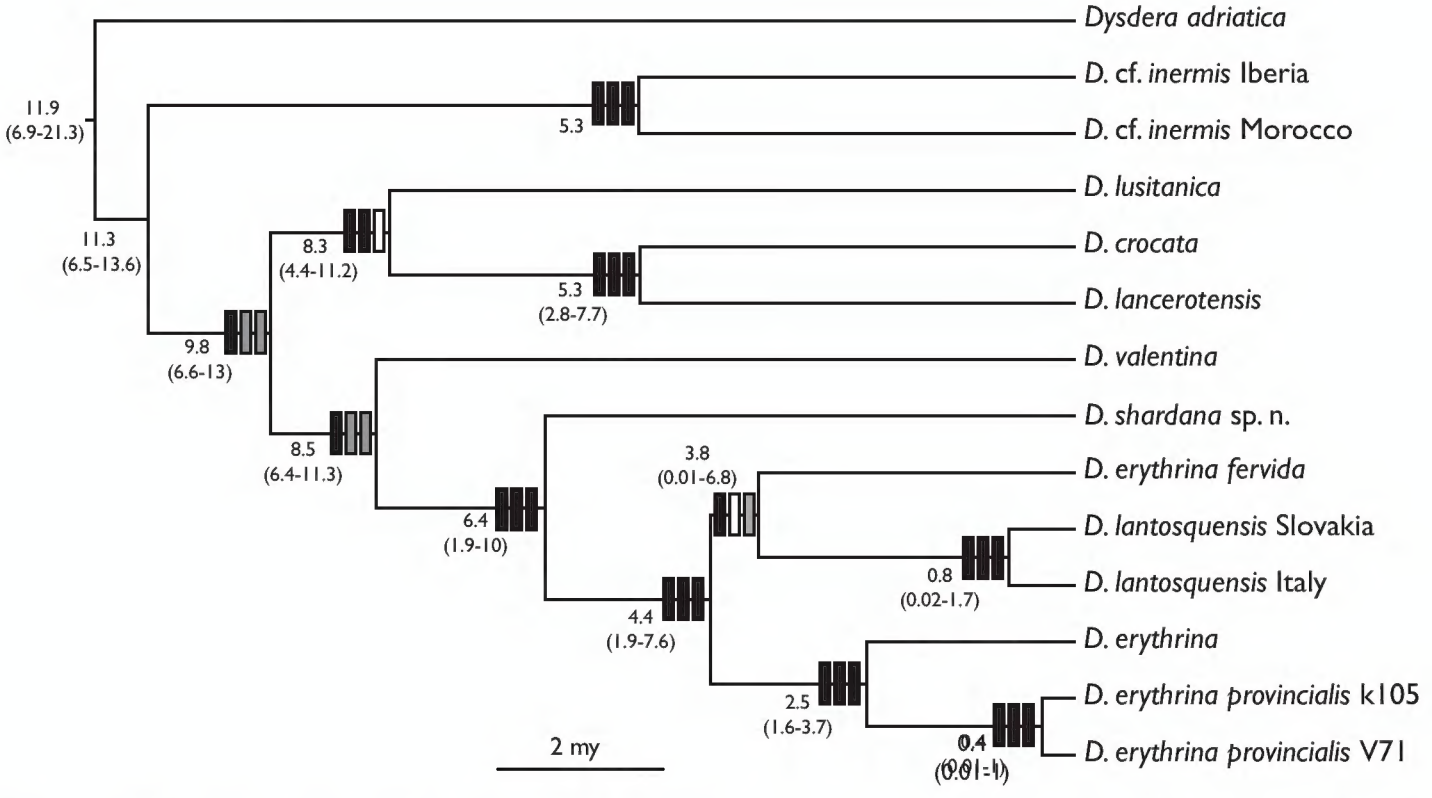


Figure 2. Chronogram of the species sampled in the present study, based on the preferred Bayesian tree obtained by simultaneous analyses of the *cox1, 16S, nad1*, 28S and H3 partitions. Bars on branches denote support as follows: anterior bar refers to posterior probability, middle bar to maximum likelihood bootstrap support, posterior bar to parsimony jackknife support. Black bar: posterior probability >0.95 or ML bootstrap, Parismony jackknife > 70, grey bar: posterior probability <0.95 or ML bootstrap, Parsimony jackknife <70, white bar: this particular clade was not recovered in the analyses. Numbers on branches are the age of the clade in millions of years before the present, estimated using penalized likelihood (set penalty=add, smoothing=3200), number in brackets refer to bootstrap confidence intervals.

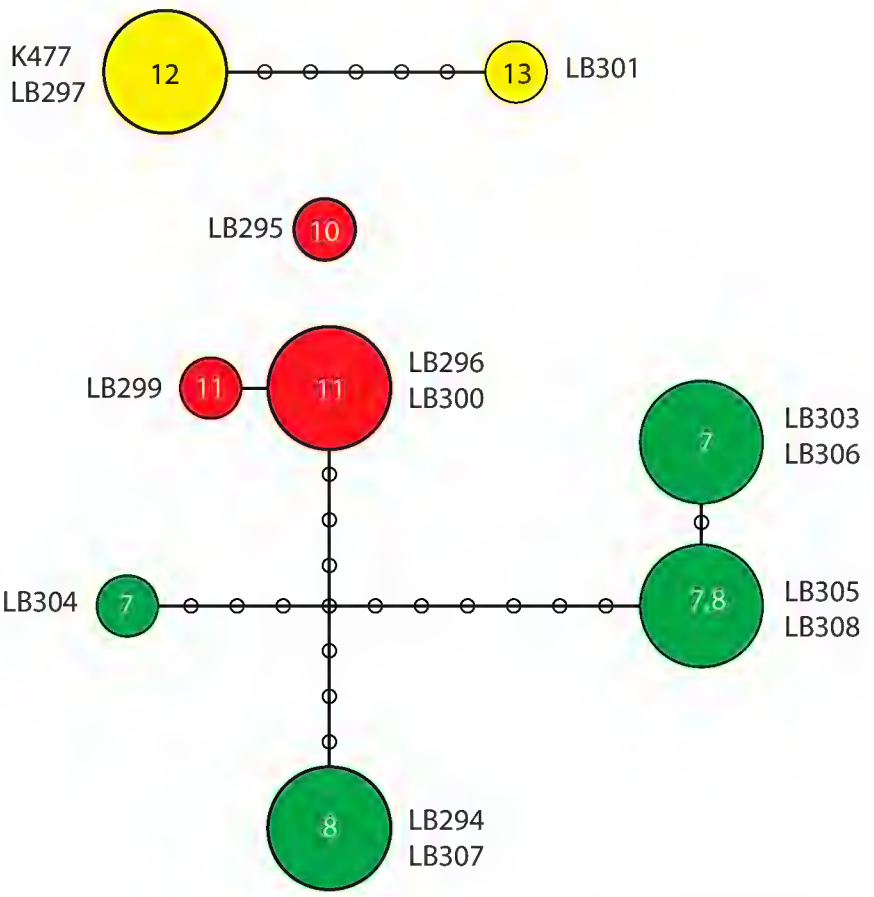


Figure 3. Haplotype network constructed using statistical parsimony (95%). Dots on branches refer to missing haplotypes. Node size proportional to number of haplotypes (1 or 2), nodes colour code and numbers inside refer to localities in Fig. 1.

yielded a single tree of 1800 steps, which mirrored results of the combined data set except for the position of *D. valentina*, which was sister to the *D. lancerotensis*+*D. crocata* clade (JS=60). The analysis of the 28S gene fragment yielded 5 trees of 136 steps, the strict consensus of which supports the sister group relationship of *D. valentina* and the *erythrina* group, but does not resolve the position of *D. shardana* sp. n. In addition, *D. lantosquensis* formed a clade with *D. erythrina* and *D. erythrina provincialis*, although with low support (JS=62). The histone 3 fragment yielded a single tree of 63 steps. The *erythrina* group, *D. shardana* sp. n. excluded, was recovered as a clade (JS=60), but the haplotype k105 of *D. erythrina provincialis* grouped with *D. fervida* (JS=61). Lineage age estimates are also summarized in Fig. 2. The split of the ancestor of *D. shardana* sp. n. from the remaining species of the *erythrina* group is estimated at 6.4 m.a. although confidence intervals recovered are very wide (from 1.9 to 10 m.a.). The fourteen *D. shardana* sp. n. individuals sequenced for the *cox1* fragment resulted in 9 unique haplotypes. Statistical parsimony analysis of these resulted in two separate networks (Fig. 3) with strong geographic structure. The first network included the haplotypes sampled from Mt. Arci and the second network connected the haplotypes from Mt. Ferru, on the one hand, and those from Gennargentu, on the other. One specimen from Mt. Ferru remains isolated from any of the networks. Uncorrected genetic distances for the *cox1* gene between *D. shardana* haplotypes and between and within haplotypes of the species related to *D. shardana* sp. n. are summarized in Table 2. Mean genetic distance between *D. shardana* sp. n. haplotypes (3.8%) was very similar to those observed in closely related species (4.6% and 3.4%. for *D. lantosquensis* and *D. erythrina provincialis*, respectively).

Table 2. Uncorrected genetic distances of the cox1 gene between *D. shardana* sp. n. haplotypes (above line) and between and within closely related species (below line).

	LB303	LB305	LB294	LB304	LB299	LB296	LB295	K477	*D. shardana* sp. n.	*D. lantosquensis*	*D. e. fervida*	*D. e. provincialis*	*D. erythrina*
LB305	0.003												
LB294	0.019	0.016											
LB304	0.019	0.016	0.012										
LB299	0.021	0.018	0.014	0.014									
LB296	0.02	0.017	0.012	0.012	0.002								
LB295	0.028	0.031	0.023	0.02	0.031	0.03							
K477	0.065	0.064	0.065	0.061	0.07	0.07	0.069						
LB301	0.071	0.07	0.071	0.067	0.076	0.076	0.075	0.009					
D. shardana sp. n.									0.038				
D. lantosquensis									0.167	0.046			
D. e. fervida									0.157	0.15			
D. e. provincialis									0.161	0.147	0.146	0.034	
D. erythrina									0.168	0.164	0.172	0.13	
D. valentina									0.174	0.187	0.176	0.173	0.169

Taxonomy

Dysdera Latreille, 1804

Dysdera Latreille, 1804, type species *Aranea erythrina* Walckenaer, 1802: 224 (unspecified sex) by original designation, unspecified number of syntype specimens from France, surroundings of Paris (C. A. Walckenaer), repository unknown, supposedly lost.

Diagnosis. See Deeleman-Reinhold and Deeleman (1988).

Species included. The genus presently includes 248 species (Platnick 2009).

Dysdera jana Gasparo & Arnedo, sp. n.

urn:lsid:zoobank.org:act:A4417862-62F2-433B-BCC9-E677576FF1C9

Figs 4, 6, 8, 10-11, 14-18

Type material. Holotype: male: Italy, Sardinia, Nuoro, Dorgali, along Viale J.F. Kennedy (=SS 125), 450 m, under *Pinus*, 17.X.1989; F. Gasparo (MSNVR AR 0010).

Paratypes: 1 male (CRBA001736) and 4 females (CRBA001736-1737, MSNVR AR 0011-12) same data and collector.

Additional material examined. Nuoro: 2 km S of Dorgali, 500 m, under *Quercus ilex*, F. Gasparo leg. 20.X.1989, 2 ♀♀ (CG); Supramonte di Oliena, 900 m, under *Quercus ilex*, F. Gasparo leg. 13.X.1989, 2 ♀♀ (CG); Sa Oche (km 7 ESE of Oliena), 150 m, under *Quercus ilex*, F. Gasparo leg. 16.X.1989, 4 ♀♀ (CG).

Diagnosis. *Dysdera jana* sp. n. can be distinguished from continental species of the *erythrina* group by the shape of the posterior-distal part of the tegulum, which is expanded in the continental species. It can also be distinguished from *D. erythrina* and *D. erythrina provincialis* by the lateral concave shape of the chelicera and a heavily wrinkled (foveate) carapace (Figs 4, 6). In addition, continental females of the *erythrina* group exhibit a characteristic hourglass-shaped spermatheca, which is very attenuated in *D. jana* sp. n. (Fig. 11). It differs from the other Sardinian species of the *erythrina* group by the spineless dorsal tibia 4, the position and length of the finger-like process on the tip of the male bulb (compare Figs 15 and 21), and by the fusion of the anterior sclerotised stripes of the vulva VA (compare Figs 11 and 13).

Etymology. The name in apposition refers to the "Domus de janas", characteristic Sardinian tombs dug in the rock often according to a labyrinth architecture. Domus de Jana translates in Sardinian as "House of the fairies or of the witches". It also happens to be the name of the first author's goddaughter.

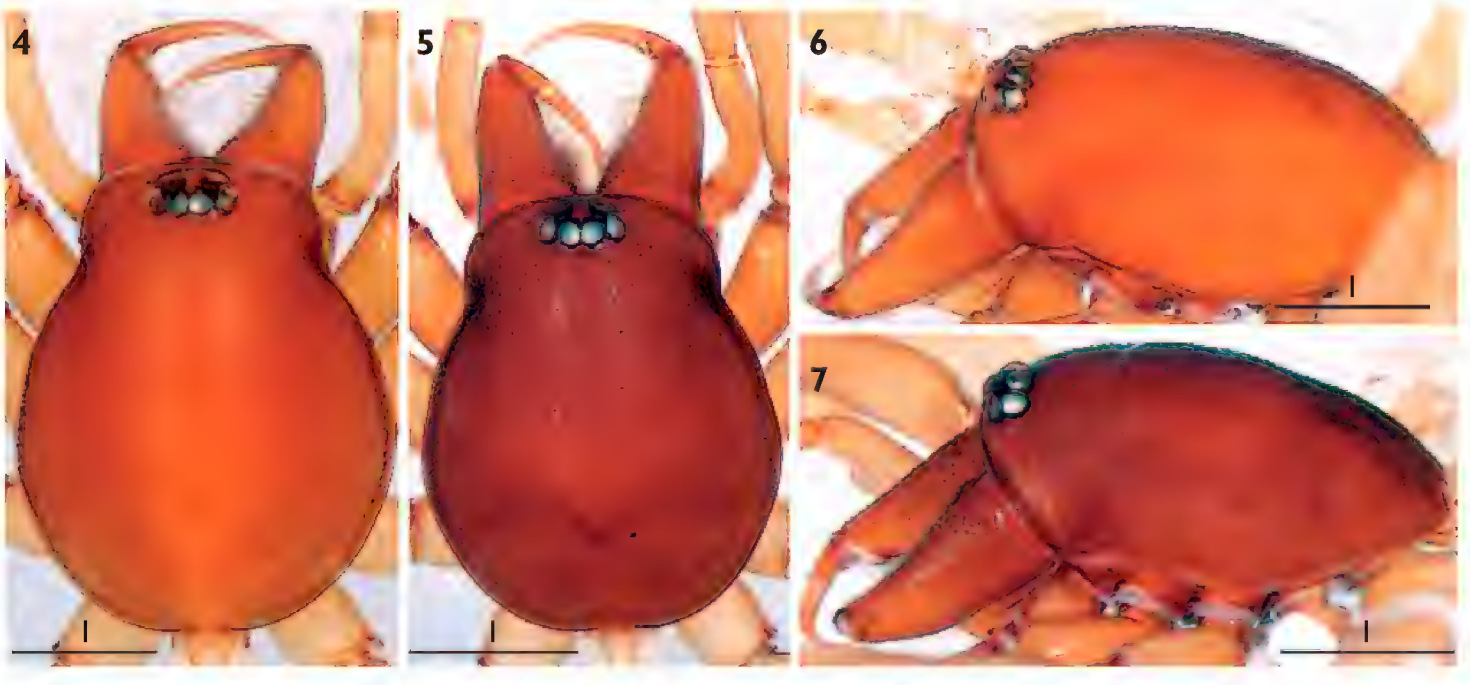
Description. *Holotype male* num. CRBA 1736. Figs 4, 6, 8, 14-18. Carapace (Fig. 4) 2.6 mm long; maximum width 2.09 mm; minimum width 1.3 mm. Orange, darkened at borders; foveate at borders, slightly wrinkled at middle, covered with small black grains. Frontal border roughly round, from 1/2 to 3/5 carapace length; anterior lateral borders parallel; rounded at maximum dorsal width, back lateral borders

straight; back margin wide, straight. AME diameter 0.17 mm; PLE 0.14 mm; PME 0.12 mm; AME slightly back from frontal border, separated from one another by about 1/2 diameter, close to PLE; PME very close to each other, about 1/3 PME diameter from PLE. Labium trapezoid-shaped, base wider than distal part; longer than wide at base; semicircular groove at tip. Sternum orange, darkened on borders; wrinkled; uniformly covered in slender black hairs.

Chelicerae 1.17 mm long, about 2/5 of carapace length in dorsal view; fang medium-sized, 0.97 mm; basal segment proximal dorsal, ventral side scantly covered with piligerous granulations; concave-shaped in lateral view (Fig. 6). Chelicera inner groove medium-sized, about 2/5 cheliceral length; armed with three teeth and lamina at base; B=M>D; D triangular, slightly below groove midpoint; B close to basal lamina; M close to B. Legs yellow. Lengths of male described above: fe1 2.01 mm (all measurements in mm); pa1 1.12; ti1 1.71; me1 1.43; ta1 0.46; total 6.73; fe2 1.94; pa2 1.07; ti2 1.63; me2 1.43; ta2 0.43; total 6.5; fe3 1.5; pa3 0.82; ti3 1.07; me3 1.17; ta3 0.41; total 4.97; fe4 1.89; pa4 1.02; ti4 1.58; me4 1.63; ta4 0.48; total 6.6; fe Pdp 1.12; pa Pdp 0.56; ti Pdp 0.61; ta Pdp 0.66; total 2.96; relative length: 1>4>2>3. Spination: leg1, leg2 spineless. Fe3d spineless; pa3 spineless; tb3d spines arranged in one band; distal 1.0.0; tb3v with one terminal spine on prolateral margin. Fe4d spineless; pa4 spineless; tb4d spines spineless; tb4v spines arranged in one band; proximal 0.1.0; with one terminal spine at one leg and two on other one. Dorsal side of frontal legs, ventral side of palp covered with hairs. Claws with eight teeth or less; hardly larger than claw width.

Abdomen 4.13 mm long; cream-coloured; cylindrical. Abdominal dorsal hairs 0.05 mm long; thin, curved, not compressed, pointed; uniformly, thickly distributed.

Male copulatory bulb (Fig. 8) T slightly shorter than DD; external, internal distal borders sloped backwards. DD slightly bent in lateral view, clearly less than 45°; internal distal border not expanded. ES wider, more sclerotised than IS; IS continuous to



Figures 4-7. Carapace, dorsal view: **4** *D. jana* sp. n. holotype **5** *D. shardana* sp. n. holotype. Carapace, lateral view: **6** *D. jana* sp. n. holotype **7** *D. shardana* sp. n. holotype.

tip. DD tip (12-15) straight in lateral view; AR present, parallel to DD; finger-like projection at distal end of the AR ridge base, as long as AR length. C absent. AC absent. LF absent. L reduced to distal part. LA absent. F absent. AL absent. P (Fig. 18) fused to T; perpendicular to T in lateral view; lateral length about 1/4 of T width; ridge present, perpendicular to T; not expanded, upper margin smooth; distally slightly projected.

Paratype female num. CRBA 1736. Figs 10-11. All characters as in male except: Carapace 3.26 mm long; maximum width 2.7 mm; minimum width 1.76 mm. Brownish orange, darkened at borders; slightly foveate at borders, wrinkled at middle, covered with tiny granulations. AME diameter 0.19 mm; PLE 0.18 mm; PME 0.16 mm; AME slightly back from frontal border, separated from one another by about 2/3 diameter, close to PLE; PME very close to each other, about 2/5 PME diameter from PLE. Sternum orange, darkened on borders; very slightly wrinkled, mainly between legs and frontal border.

Chelicerae 1.5 mm long, fang long, 1.33 mm; basal segment proximal dorsal, ventral side scantly covered with piligerous granulations. Legs orange. Lengths of female described above: fe1 2.5 mm (all measurements in mm); pa1 1.56; ti1 2.04; me1 1.84; ta1 0.48; total 8.42; fe2 2.35; pa2 1.48; ti2 1.94; me2 1.79; ta2 0.51; total 8.06; fe3 1.84; pa3 1.07; ti3 1.33; me3 1.58; ta3 0.51; total 6.32; fe4 2.35; pa4 1.33; ti4 1.89; me4 2.24; ta4 0.56; total 8.36; fe Pdp 1.43; pa Pdp 0.66; ti Pdp 0.77; ta Pdp 0.79; total 3.65; relative length 1>4>2>3. Spination: tb3d spines arranged in two bands; proximal 0-1.0.0; distal 1.0.0; tb3v spines arranged in one band; proximal 0.1.0; with two terminal spines. Tb4d spines spineless; tb4v spines arranged in one band; proximal 0.1.0; with two terminal spines.

Abdomen 3.73 mm long. Abdominal dorsal hairs 0.07 mm long; thin, curved, not compressed, pointed; uniformly, thickly distributed. Vulva DA (Fig. 10) clearly distinguishable from VA; DA slightly wider than long; DF wide in dorsal view. MF margins not fused, poorly developed, membranous. VA rectangle-like; frontal region completely sclerotized (Fig. 11); posterior region internal margin sclerotized, forming two stripes fused at the anterior part, diverging backwards; AVD absent. S attachment not projected under VA; arms as long as DA, slightly curved; tips not projected; neck as wide as arms. TB usual shape.

Variation. Carapace ranges in length from 2.37 mm to 3.77 mm, males (n=2) 2.42-2.60, female (n=10) average 3.14 (2.37-3.77). Females from Supramonte di Oliena are significantly smaller (2.37-2.72) than those from Dorgali and Sa Oche. Male (n=2) leg spination reduced compared to females. Spination variability summarised in Table 3.

Distribution. Known from several localities around the Supramonte region, a mountain and highland range in Sardinia, lying in the north-western part of the Gulf of Orosei.

Table 3. Intraspecific spination variability in *D. jana* sp. n.

	Proximal	**Medio-proximal**	**Medio-distal**	**Distal**
Tibia 3 dorsal	0-1.0.0	0	0	1.0.0
Tibia 4 dorsal	0	0	0	0
Tibia 3 ventral	0	0	0	1.0.0
Tibia 4 ventral	0.1.0	0	0	1.0.1

Figures 8-9. Left male palp, retrolateral view: **8** *D. jana* sp. n. holotype **9** *D. shardana* sp. n. holotype.

***Dysdera shardana* Opatova & Arnedo, sp. n.**
urn:lsid:zoobank.org:act:0D4FFE8A-AA79-4F64-9420-C9AC902A612A
Figs 5, 7, 9, 12-13, 19-22

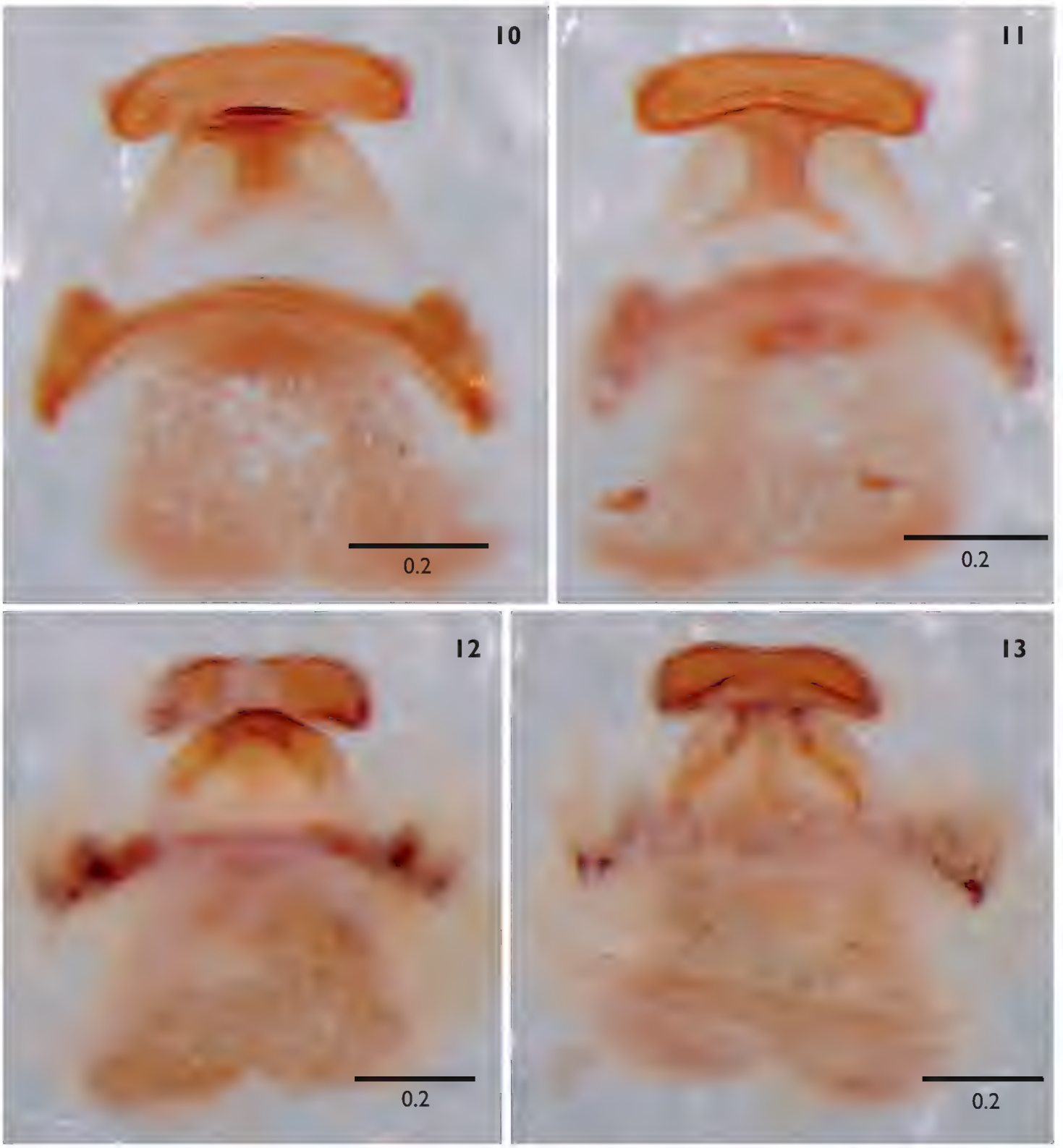
Dysdera kollari Grasshoff, 1959: 219, figs 6 [♂] (NO Torralba, SO Cuglieri, S Cuglieri).
Schult, 1983: 69-84, fig. 8 [♂] (same specimens as Grasshoff, 1959). Misidentification.

Type material. Holotype: male: Italy, Sardinia, Oristano, Morgongiori, Is Benas recreation area, Mt. Arci, 39.7606N, 8.76062E, 566 m, mixed *Pinus* and *Quercus* forest, 15.X.2005, M. Arnedo and M. Mejía-Chang leg. (CRBA001246).

Paratypes: 1 female (CRBA001246), 1 female (CRBA001247, DNA extraction K477), 2 juvs (CRBA001713, CRBA001714, DNA extraction LB297) same data and collectors.

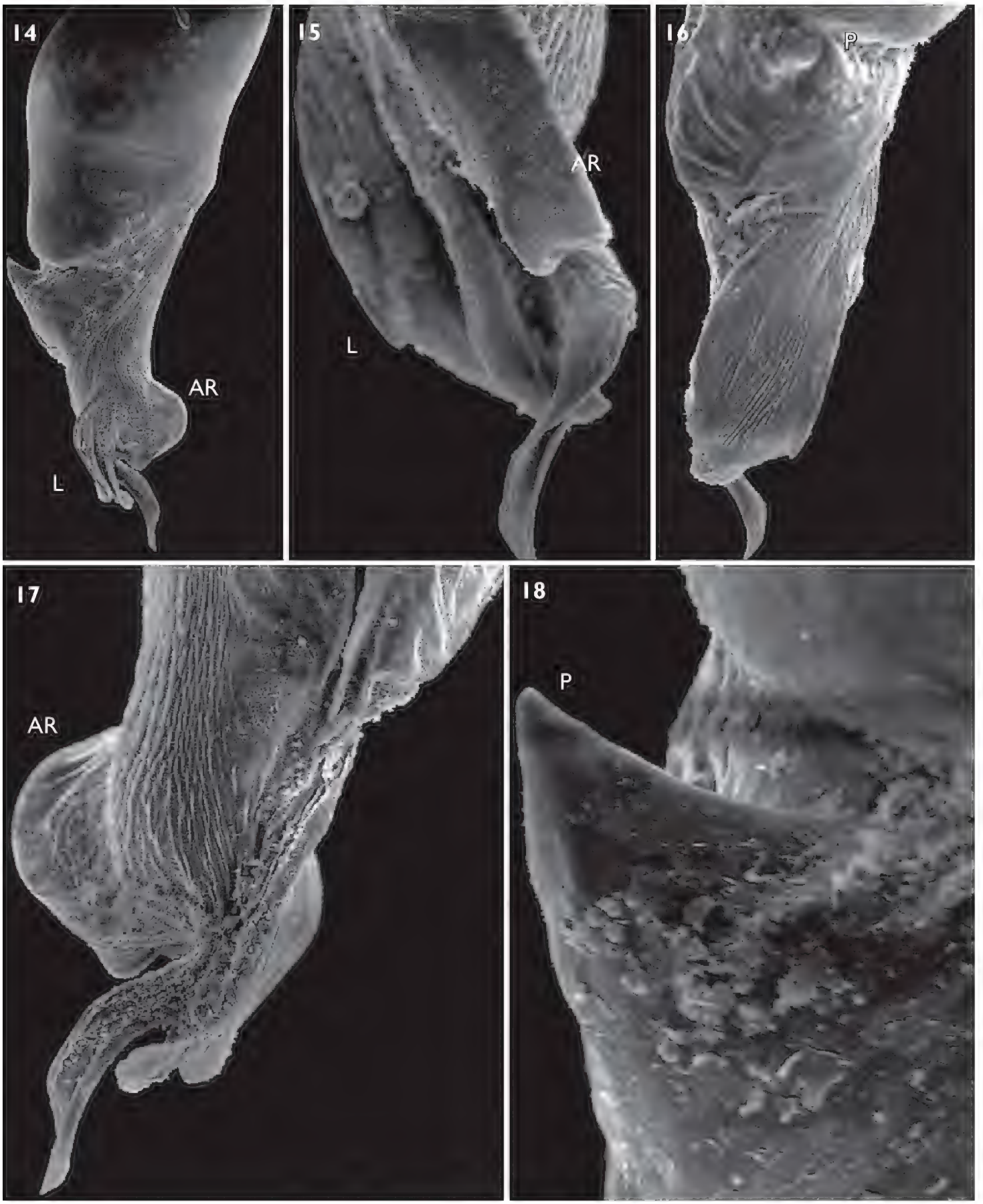
Additional material examined. Italy: Sardinia: **Cagliari**: Fluminimaggiore, Is Arenas, under *Quercus ilex*; 800 m; G. Gardini leg. 29.III.1991, 1 ♂ (CG); Flumini-

maggiore, Tempio di Antas, under *Quercus ilex*; 360 m; G. Gardini leg. 29.III.1991, 1 ♀ (CG). **Nuoro**, Aritzo, Castagneto Geratzia, Mt. Gennargentu; 39.96846N 9.19782E; 867 m; *Castaneus* and *Quercus suber* forest; 17.X.2005; M. Arnedo, M. Mejía-Chang & G. Giribet leg. (1♀ CRBA001262 cryo-collection, DNA extraction LB303, 1♀ CRBA001710 cryo-collection, DNA extraction LB304, 1♂ CRBA0001263 cryo-collection, DNA extraction LB305, 1♀ CRBA0001711 cryo-collection, DNA extraction LB306); Desulo, rd. to Fonni, Mt. Gennargentu; 40.05645N 9.22955E; 1146 m; *Quercus pubens* forest; 17.X.2005; M. Arnedo, M. Mejía-Chang and G. Giribet leg. (1♂ CRBA0001268, DNA extraction LB294, 1♂ CRBA001270 cryo-collection. DNA extraction LB307, 1♂ CRBA001712 cryo-collection. DNA extraction LB308);



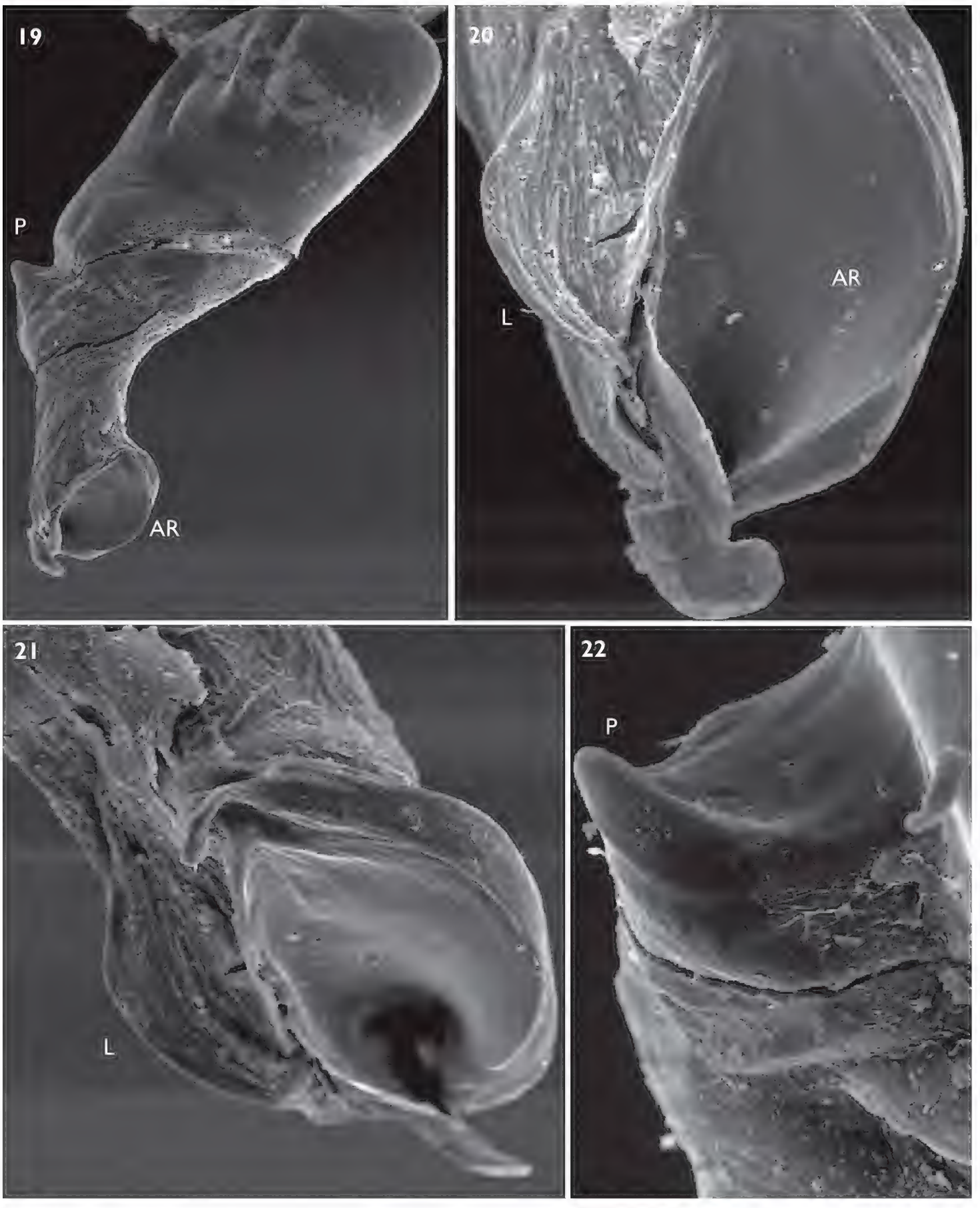
Figures 10-13. *D. jana* sp. n. paratype female **10** vulva, dorsal view **11** vulva, ventral view. *D. shardana* sp. n. paratype female **12** vulva, dorsal view **13** vulva, ventral view.

Macomer, Mt. S. Antonio, A. Vigna leg. 2.V.1967, 1 ♂ (MCVR). **Oristano**, Cuglieri, rd. to San Leonardo 7 Fuentes, Mt. Ferru; 40.17931N 8.58544E; 587 m; dry, open *Quercus* forest; 14.X.2005; M. Arnedo and M. Mejía-Chang leg. (1♀ CRBA001238, cryo-collection, DNA extraction LB295); nearby S. Leonardo 7 Fuentes, Mt. Ferru, 40.20362N 8.68969E; 699 m; old crops and open forest *Quercus* and *Rubus*; 14.X.2005; M. Arnedo & M. Mejía-Chang leg. (1♀ CRBA001240 cryo-collection,



Figures 14-18. *D. jana* sp. n. holotype, right male bulb **14** prolateral view, general **15** anterior view **16** posterior view **17** retrolateral view **18** P, prolateral view.

DNA extraction LB296, 1♀ CRBA001707 cryo-collection, DNA extraction LB299, 1juv. CRBA001708 cryo-collection, DNA extraction LB300,); Morgongiori, Bruncu Mutzuis, 39.77145N 8.74666E; 761 m; open *Quercus* forest w/ mosses; 15.X.2005; M. Arnedo & M. Mejía-Chang leg. (1sub♂ CRBA001256, cryo-collection, DNA extraction LB301)



Figures 19-22. *D. shardana* holotype, right male bulb **19** prolateral view, general **20** prolateral view, tip detail **21** anterior view **22** P, prolateral view.

Diagnosis. *Dysdera shardana* sp. n. differs from continental species of the *erythrina* group in the shape of the posterior-distal part of the tegulum, which is expanded in the continental species. In addition, continental females of the *erythrina* group exhibit a characteristic hourglass-shaped spermatheca, which is very attenuated in *D. shardana* sp. n. It can also be distinguished from *D. erythrina* and *D. erythrina provincialis* by the lateral concave shape of the chelicera and a heavily wrinkled (foveate) carapace (Figs 5, 7). It differs from *D. jana* sp. n., also from Sardinia, in the presence of dorsal spines on tibia 4, the position and length of the finger-like process on the tip of the male bulb (compare Figs 15 and 21), and by the fusion of the anterior sclerotised stripes of the vulva VA (compare Figs 9 and 11).

Etymology. The name in apposition refers to the Shardana people, one of several groups of "Sea Peoples" that appear in fragmentary historical records (Egyptian inscriptions) for the Mediterranean region in the second millennium B.C. According to some scholars they settled in Sardinia after the period of Ramses III (around 1180 B.C.).

Description. *Holotype male* num. CRBA 1246. Figs 5, 7, 9, 19-22. Carapace (Fig. 5) 3.01 mm long; maximum width 2.35 mm; minimum width 1.68 mm. Dark red, darkened at borders; foveate at borders, slightly wrinkled in the middle, covered with small black grains. Frontal border roughly round, from 1/2 to 3/5 carapace length; anterior lateral borders parallel; rounded at maximum dorsal width, back lateral borders straight; back margin wide, straight. AME diameter 0.18 mm; PLE 0.18 mm; PME 0.16 mm; AME slightly backwards from frontal border, separated from one another by about 2/3 diameter, close to PLE; PME close to each other, less than 1/4 PME diameter from PLE. Labium trapezoid-shaped, base wider than distal part; longer than wide at base; semicircular groove at tip. Sternum brownish orange, darkened on borders; wrinkled; uniformly covered in slender black hairs.

Chelicerae 1.2 mm long, about 2/5 of carapace length in dorsal view; fang long, 1.22 mm; basal segment proximal dorsal, ventral side scantly covered with piligerous granulations; concave-shaped in lateral view (Fig. 7). Chelicera inner groove long, about 1/2 cheliceral length; armed with three teeth and lamina at base; B=M>D; D triangular, slightly below groove midpoint; B close to basal lamina; M close to B. Legs yellow. Lengths of male described above: fe1 2.3 mm (all measurements in mm); pa1 1.3; ti1 1.84; me1 1.66; ta1 0.48; total 7.57; fe2 2.09; pa2 1.22; ti2 1.71; me2 1.58; ta2 0.51; total 7.11; fe3 1.58; pa3 0.87; ti3 1.07; me3 1.43; ta3 0.41; total 5.31; fe4 2.09; pa4 1.17; ti4 1.68; me4 1.89; ta4 0.51; total 7.34; fe Pdp 1.22; pa Pdp 0.66; ti Pdp 0.66; ta Pdp 0.71; total 3.26; relative length: 1>4>2>3. Spination: leg1, leg2 spineless; fe3d spineless; pa3 spineless; tb3d spines arranged in two bands; proximal 1.0.0; distal 1.0.0; tb3v spines arranged in one band; proximal 0.1.0; with one prolateral spine on left leg, and two terminal on right leg. Fe4d spineless; pa4 spineless; tb4d spines arranged in two bands; proximal 1.0-1.1; distal 0.0.0-1; tb4v spines arranged in one band; proximal 0.1.0; with two terminal spines. Dorsal side of frontal legs, ventral side of palp covered with hairs. Claws with eight teeth or less; hardly larger than claw width.

Abdomen 3.26 mm long; cream-coloured; cylindrical. Abdominal dorsal hairs 0.13 mm long; thin, curved, not compressed, pointed; uniformly thickly distributed.

Male copulatory bulb (Fig. 9) T slightly shorter than DD; external, internal distal border sloped backwards. DD bent about 45° in lateral view; internal distal border not expanded. IS, ES equally developed; IS truncated at DD middle part. DD tip (Figs 19-21) straight in lateral view; AR present, parallel to DD; finger-like distal projection at AR retrolateral side, shorter than AR length. C absent. AC absent. LF absent. L reduced to distal part. LA absent. F absent. AL absent. P (Fig. 22) fused to T; sloped forming an angle of about 135° to T in lateral view; lateral length about 1/4 of T width; ridge present, parallel to T; not expanded, upper margin smooth; not distally projected; back margin slightly folded towards internal side.

Paratype female num. CRBA 1246. Figs 12-13. All characters as in male except: Carapace 3.19 mm long; maximum width 2.55 mm; minimum width 1.68 mm. AME diameter 0.18 mm; PLE 0.19 mm; PME 0.16 mm; AME slightly back from frontal border, separated from one another by about 2/3 diameter, touching PLE; PME very close to each other, less than 1/4 PME diameter from PLE. Sternum very slightly wrinkled, mainly between legs and frontal border.

Chelicerae 1.38 mm long; fang 1.48 mm; basal segment proximal dorsal side scantly covered with piligerous granulations. Chelicera armed with three teeth and lamina at base; M>B=D. Legs orange. Lengths of female described above: fe1 2.22 mm (all measurements in mm); pa1 1.35; ti1 1.73; me1 1.58; ta1 0.46; total 7.34; fe2 2.04; pa2 1.28; ti2 1.68; me2 1.53; ta2 0.48; total 7.01; fe3 1.66; pa3 0.92; ti3 1.15; me3 1.38; ta3 0.41; total 5.51; fe4 2.27; pa4 1.22; ti4 1.79; me4 1.89; ta4 0.48; total 7.65; fe Pdp 1.17; pa Pdp 0.61; ti Pdp 0.61; ta Pdp 0.71; total 3.11; relative length 4>1>2>3. Spination: tb3d spines arranged in two bands; proximal 1.0.0; distal 1.0.0; tb3v with two terminal spines. Tb4d spines arranged in one band; proximal 1.0.1; tb4v spines arranged in one band; proximal 0.1.0; with two terminal spines.

Abdomen 4.56 mm long. Abdominal dorsal hairs 0.14 mm long; medium-sized, curved, compressed, pointed; uniformly, thickly distributed. Vulva (Figs 12-13) DA clearly distinguishable from VA; DA slightly wider than long; DF wide in dorsal view. MF margins not fused, poorly developed, membranous (Fig. 12). VA rectangle-like, pointed expansion at middle frontal part (Fig. 13); frontal region completely sclerotized; posterior region internal margin sclerotized, forming two divergent stripes; AVD absent. S attachment not projected under VA; arms as long as DA, slightly curved; tips not projected; neck hardly visible. TB usual shape.

Variation. Carapace ranges in length from 2.50 mm to 3.42 mm, males (n=7) average 2.79 (2.50-3.06), female (n=8) average 3.07 (2.68-3.42). In general male legs

Table 4. Intraspecific spination variability in *D. shardana* sp. n.

	Proximal	**Medio-proximal**	**Medio-distal**	**Distal**
Tibia 3 dorsal	1.0.0	0	0	1.0.0
Tibia 4 dorsal	0-1.0-1.1	0	0	0.0.0-1
Tibia 3 ventral	0. 0-1.0	0	0	1.0.0-1
Tibia 4 ventral	0. 0-1.0	0	0	1.0. 0-1

bear fewer spines that female legs, one male form Mt. S. Antonio representing an extreme case of reduced spination. Spination variability summarised in Table 4.

Distribution. Known from several localities around the western half of Sardinia, and also in the Gennargentu region on the central-eastern side of the island, spanning from 360 to above 1100 m elevation.

Discussion

Molecular phylogenetic analyses unambiguously support the close relationship of *Dysdera shardana* sp. n. with the species of the *erythrina* group. Morphological synapomorphies for this clade include a large, semicircular expansion (AR) at the DD tip, running parallel to the bulb proximal-distal axis and a bilobular, granulated posterior diverticulum in the female vulva. Both *D. shardana* sp. n. and *D. jana* sp. n. exhibit a regular-shaped tegulum. This suggests that the particular shape of the tegulum with a mid-part constraint and an expansion around the P region is most likely a synapomorphy of the clade formed by *D. lantosquensis* and *D. erythrina*. The *erythrina* group was not defined in cladistic terms and hence it is not possible to assess its limits. The taxonomic sampling of the phylogenetic analysis conducted in this study included two species closely resembling those originally proposed to be part of the *erythrina* group, namely *D.* cf. *inermis* (closely similar to *D. fuscipes)* and *D. lusitanica* (resembling *D. veigai*). The phylogenetic analyses revealed that they are not closely related to *D. erythrina*. Deeleman-Reinhold and Deeleman (1988) did not justify the inclusion of *D. fuscipes* and *D. veigai* species into the *erythrina* group and thus it is difficult to evaluate convergence in morphological traits. All these species, however, share a particular shape of the P, which is in most cases short and with the proximal margin parallel, instead of perpendicular, to the proximal-distal axis of the bulb. Close examination of this structure, however reveals, obvious differences among the species (e.g., the particular shape observed in the *D. lantosquensis* / *D. erythrina* clade is probably synapomorphic for this group). The eastern Iberian species *D. valentina* was included in the analyses based on the similarities in the distal part of the male bulb DD to *D. erythrina*. All analyses support (although only marginally under parsimony and maximum likelihood) the sister group relationship of *D. valentina* with the *D. shardana* sp. n. / *D. erythrina* clade, suggesting that the presence of a long, arch-like expansion on the anterior-distal side of the DD, running parallel to a short L (see Ribera 2004, Figs 1A-B) could constitute a synapomorphy of a more inclusive clade that would also embrace the *erythrina* group. More morphological comparative data and new phylogenetic analyses based on more exhaustive taxonomic sampling would be required to confirm the latter suggestion.

Based on morphological evidence, there is little doubt that the two new Sardinian *Dysdera* species are closely related. They are extremely similar in somatic morphology (some minor differences in leg spination) and share at least one exclusive character: the presence of a finger-like projection at the tip of the male bulb (although it is not completely clear whether the process actually grows from the same position, see diagnosis).

Unfortunately, the lack of genetic data for *D. jana* sp. n. precludes discerning whether the two endemics are sister species or whether one of them is actually more closely related to the *D. erythrina* /*D. lantosquensis* clade. Examples of local diversification in Sardinia have been reported in other ground-dwelling animals. The cave beetle genera *Ovobathysciola* and *Patriziella* form a monophyletic group that includes six endemic species in Sardinia. Molecular clock analysis suggests that separation among the *Ovobathysciola* / *Patriziella* species started about 16 to 10 m.a., assuming that the clade originated as a result of the split of the Corsico-Sardinian plate from the Iberian peninsula (~30 m.a.) (Caccone and Sbordoni 2001). The *Hydromantes* cave salamanders, on the other hand, include five endemic lineages in Sardinia, which have been recently shown to form a paraphyletic group with regard to closest relatives in south-eastern France and northern-central Italy (Carranza et al. 2008). In this case, molecular time estimates suggest that the Messinian Salinity Crisis facilitated the two independent colonisation events that gave rise to present day Sardinian endemics (although a single colonisation of the island followed by a back colonisation of the mainland is an equally parsimonious explanation). The unresolved phylogenetic position of *D. jana* sp. n. hinders distinguishing between a scenario of a single colonisation of Sardinia followed by in situ speciation of the two new *Dysdera* species from a scenario with a colonisation of Sardinia by the common ancestor of the *D. shardana* sp. n. / *D. erythrina* clade followed by back colonisation of the mainland. Regardless of the scenario, time estimates clearly suggest that the split of *D. shardana* sp. n. from mainland relatives post-dates the opening of the western Mediterranean basin. Time estimates of the split of *D. shardana* sp. n. and of the diversification of the *D. erythrina* / *D. lantosquensis* clade are close enough to the Messinian Salinity Crisis time period to suggest that this geological event may have played a role in the origin of these lineages, as has also been proposed for the harvestmen genus *Trogulus* (Schönhofer and Martens 2008). Confidence intervals of the time estimates, however, are too large to rule out other alternative scenarios. Moreover, it has been shown based on mitochondrial substitution rates that trans-Gibraltar lineage divergences in the scorpion *Buthus occitanus* predate the opening of the strait of Gibraltar (Gantenbein and Largiader 2003), which opens the door to the possibility that our calibration point is too young and hence the time of colonization of Sardinia is an underestimation.

At this stage, it is not clear what factors may have prompted the speciation of the two new Sardinian endemics. The two species show allopatric distribution, generally associated to middle (500 m) and high elevations (1000 m). *D. jana* sp. n. seems to have a narrow distribution, restricted to the Supramonte region, while *D. shardana* sp. n. is widespread across most of the islands, except for the northern and southern-most areas (Mt. Limbara and Sette Fratelli, respectively). Interestingly, species of the cave-dwelling beetle lineage *Ovobathysciola* / *Patriziella* show a very similar geographic distribution pattern to *D. jana* sp. n. (*O. graffiti* is endemic to the Supramonte region) and *D. shardana* sp. n. (the rest of the *Ovobathysciola* / *Patriziella* species are found in Mt. Arci, Mt. Ferru and Gennargentu), although with contrasting levels of species diversity and phylogenetic relationships (compare fig. 2 in Caccone and Sbordoni 2001

with our Fig. 3). Genetic analysis of several *D. shardana* sp. n. populations, however, revealed strong phylogeographic patterns in this species. Populations from Mt. Arci form an independent network (i.e., highly divergent haplotypes) from those sampled from Mt. Ferru and Gennargentu, which are also separated (the southernmost known populations on Sulcis-Iglesiente could not be analysed genetically). This observation does not necessarily imply that the former haplotype groups should be considered independent evolutionary lineages, but it does suggest that there are environmental factors (e.g., unsuitable lowland habitat due to warmer climatic conditions) restricting gene flow across geographic areas separated by just 50 km. Environmental restrictions to gene flow could eventually lead to speciation and hence explain the presence of two sibling species of the *erythrina* group on Sardinia.

Conclusion

Two new species of the woodlouse-hunter spider genus *Dysdera* are described from Sardinia. Phylogenetic evidence and morphological features suggest that they are closely related to the type species of the genus *D. erythrina*. Time estimates reject an Oligocene origin of the new *Dysdera* species and instead suggest that the Messinian Salinity Crisis shaped the diversification of Sardinian endemics and their closest relatives. Deep genetic divergences and population structure in *Dysdera shardana* sp. n. identifies restriction to gene flow due to environmental factors as the most plausible explanation for local speciation events.

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